Tetrahedron Letters,Vol.30,No.12,pp 1479-1482,1989 0040-4039/89 $3.00 + .00
Printed in Great Britain Pergamon Press plc

THE SOLVOLYSIS OF N-ACETOXY-4-ACETYLAMINOSTILBENE: IRREVERSIBLE FORMATION OF NITRENIUM IONS

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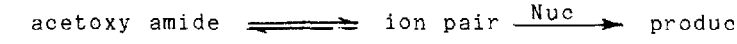
Abstract: Extensive kinetic studies and product analysis, coupled with O-18 labeling experiments have shown that the solvolysis of N-acetoxy-4-acetylaminostilbene in buffered aqueous acetone proceeds via irreversible nitrenium ion formation. The conflict between this conclusion and that of a previous study is explained

Aromatic amines and amides make up one of the most extensively studied classes of chemicals which are carcinogenic to animals(1) and man(2). It is generally accepted that the activation of these compounds involves N-hydroxylation(3-7) followed by O-esterification(8) and subsequent electrophilic attack by the ultimate metabolite on critical cellular macromolecules. The ultimate carcinogen has been postulated to be a nitrenium ion(9-11) although attack by neutral species has not been entirely ruled out.

Although numerous esters have been implicated in the carcinogenic process, the most widely studied models are the N-acetoxy-N-acetylarylamines. In 1970, Scribner et al.(10) reported that the solvolysis of N-acetoxy-N-acetyl-4-aminostilbene and other N-acetoxy-N-acetylarylamines in 40% buffered aqueous acetone involved reversible formation of nitrenium ions. This conclusion was based upon studies of the rate of release of water-soluble radioactivity, presumably acetate, from several N-acetoxy-(carbonyl-^{14}C)-N-acetylarylamines. In particular, they reported that the rate of reaction was non-linearly dependent on citrate ion- and methionine- concentrations. This was interpreted in terms of an intermediate ion pair which was capable of being intercepted by either of these nucleophiles:



The assumption was made that the rate of release of acetate ion paralleled the rate of disappearance of starting material. However, it is known that these compounds undergo rearrangement to ortho acetoxyamides, and that these intermediates further hydrolyze to release acetate, thus rendering this assumption questionable at best.

We(16) have recently shown that the solvolysis of N-acetoxy-(carbonyl-^{18}O)-N-acetyl derivatives of 1- and 2-naphthyl-, 2-fluorenyl- and 4-biphenylylamines, under conditions closely resembling those described above, involved irreversible formation of nitrenium ions. We now report the solvolysis of N-acetoxy-4-acetylaminostilbene at 40° in 40:60 acetone - aqueous buffer (pH 4.5 - 9.9), conditions identical with those used previously(10). We have measured the rate of disappearance of starting material by hplc and have found the pseudo-first-order rate constants (k_{obs}) to fit the equation:

$$k_{obs} = k_o + k_1[OH^-]$$

$k_o = (56.6 \pm 1.7) \times 10^{-6}$ sec^{-1} and $k_1 = (2.54 \pm 0.06)$mol^{-1}sec^{-1} ($r = 0.998$)

The k_1 process involves ester hydrolysis and leads solely to the corresponding hydroxamic acid. The (hydroxide-independent) k_o process dominates over the pH range 4.5 - 7.5, involves N - O bond heterolysis and leads to nitrenium ions which react with water to form α,β-dihydroxy-4-acetylaminobibenzyl (95% yield). This process is strongly dependent on solvent ionizing power (m =0.82), but is independent of common ion- or special salt-effects.

When hydroquinone or iodide ion were added to the reaction mixture, the major product became 4-acetylaminostilbene (90 - 95%), but the reaction rate remained unchanged. This is suggestive of the reduction of a nitrenium ion after the rate determining step.

The irreversibility of the ionization process indicated by our kinetic studies was also investigated by solvolyzing the starting material labelled with O-18 in the ester carbonyl. After one half-life the unreacted starting material was recovered and the location of the O-18 label was determined by mass spectrometry. The labelled starting material was prepared by reacting N-hydroxy-4-acetylaminostilbene with O-18 labelled acetyl chloride.

The fragmentation patterns of N-acetoxy-N-acetylarylamines upon electron-impact mass spectrometry involves stepwise loss of two molecules of ketene, K.

$$M^+ \longrightarrow (M - K)^+ \longrightarrow (M - 2K)^+$$

The $(M - 2K)^+$ fragment corresponds to the $(ArNHOH)^+$ ion and contains the oxygen originally bound to nitrogen. An ion of the same mass can also be formed from the rearranged ring acetoxy arylacetamide. In the present case however, rearrangement is not a significant factor, since product analysis showed that this rearrangement accounts for only 1 - 2% of the products. If we designate all products containing the isotopic label with an asterisk, then the ratio $(M^*)/(M + M^*)$ is a measure of the isotopic enrichment. We found that the isotopic purity of the starting material prior to solvolysis was 40.3%. The isotopic purity of material recovered after one half-life of solvolysis was found to be 40.6% indicating that there had been no loss of label during the reaction.

The ratio $(M - 2K)^*/(M - 2K)$ which is a measure of the extent of oxygen scrambling, was found to be 6.4% prior to solvolysis and 6.0% after reaction. This result clearly indicates that scrambling of the label did not occur during the solvolysis. The small amount of scrambling found both before and after reaction may be due to scrambling taking place either during the preparation of the starting material or while heating the sample in the mass spectrometer. The lack of increased scrambling upon solvolysis is totally consistent with the kinetic studies and indicates that the ionization is essentially an irreversible process.

In an effort to find an explanation for the conflict between the present study and that reported by Scribner et al.(10) we have repeated this study employing identical conditions to those reported earlier. In the presence of citrate ion (0.006M, pH 7.0) we found that k_{obs} was non-linearly dependent on the total methionine concentration (up to 0.06M) as was reported earlier. However we also observed that the pH of the reaction mixture decreased with increasing methionine concentration. Since the concentration of the more nucleophilic anionic methionine is pH-dependent it is essential that the pH be maintained constant. Upon repeating this experiment but adjusting the pH to 7.0 after the addition of methionone, k_{obs} was found to be <u>linearly</u> dependent on methionine concentration. In addition we found that the rate increase with increased pH was directly proportional to the concentration of the anionic form of methionine, and this was also paralleled by an increase in the production of hydroxamic acid. These data are all consistent with the hydrolysis of the ester function catalyzed by anionic methionine.

All results now indicate that the solvolysis of N-acetoxy-4-acetylaminostilbene in 40% aqueous acetone at 40^o involves the <u>irreversible</u> formation of nitrenium ions. This conflict with a previous report appears to be due to a failure to maintain constant pH in the earlier study.

Acknowledgement: We are endebted to the National Cancer Institute of the National Institutes of Health and to the Department of Energy for grants that supported this investigation.

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(Received in USA 20 October 1988)